

Internship on lumpfish production in Nordland
Rensefisk AS. Evaluation of larval development
with two commercial feeds.

Ana Cláudia Bronze Monteiro Martins



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Supervisor: Lars Jørgen Ulvan

Category: CEO of Nordland Rensefisk AS

Affiliation: Nordland Rensefisk AS, Norway

Co-Supervisor: Professor José Fernando Magalhães Gonçalves, PhD

Category: Associate Professor

Affiliation: Institute of Biomedical Sciences Abel Salazar, University of Porto

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Abstract

Norway is the world leader in salmon production, representing great economic power for the country and contributing worldwide with aquaculture fish consumption. The production of salmon like all industries has its challenges. Infestations of the most common ectoparasite, *Lepeophtheirus salmonis*, have been posing a threat to salmon health for years. Sea lice graze to salmon skin, feeds on its blood, alters fish homeostasis which creates a door for secondary infections. Consequently, immunity system becomes vulnerable, increasing the probability of diseases transmission. The presence of the parasite implies a high cost for the producers through chemical-therapeutic treatments, reduction of growth, wastage of feed and lower quality in the final product. The salmon industry has been fighting this parasite with chemical treatments in a diversity of ways. However, in recent years evidence of resistance has been reported. The high expense of producers and the negative impact on the environment, along with the new found resistance, make unacceptable the abuse of chemicals in salmon cages. Therefore a cost-effective approach to the alternative of pharmaceutical treatments is an urgent priority for the aquaculture industry. As a result co-productions with the cleaner fish have emerged. *Cyclopterus lumpus* is a recent species that has shown good efficiency in lice removal in sea cages, representing an environmentally friendly solution and reducing the financial impact. Aquaculture of *C. lumpus* has been Norway's largest bet in the last five years, yet it is species with little scientific information in terms of biology and intensive production conditions.

This work aims at the general practices of aquaculture production of lumpfish in the company Nordland Rensefisk AS in Lovund, Norway. Since the information about this species is so scarce, this master thesis was redirected into a professional internship in the production plus a trial at the larval stage. The experiment aims to evaluate the differences between two feeds: Skretting and Otohime. The test starts from hatching until the larvae reach approximately 1 g of average weight, evaluating the parameters of average weight, length and mortality rate. The Skretting proved to be the feed which enhance better the larval growth in a shorter period of time. The larval phase being the period of major vulnerability denotes that the choice of an adequate feed makes the difference in terms of growth performance and the economic impact of the company.

Resumo

A Noruega é o líder mundial de produção de salmão, que representa um grande poder económico para o país, contribuindo em todo o mundo para o consumo de peixe de aquacultura. A produção de salmão, como todas as indústrias, tem os seus desafios. As infestações do ectoparasita mais comum, *Lepeophtheirus salmonis*, representam uma ameaça à saúde do salmão há décadas. Os parasitas aderem à superfície do salmão e alimentam-se do seu muco, pele e sangue. Torna-se prejudicial quando altera a homeostasia do peixe criando uma porta para infecções secundárias. Consequentemente, o sistema imunitário torna-se vulnerável, aumentando a probabilidade de transmissão de doenças. A presença do parasita implica um custo elevado para os produtores pelos tratamentos químico-terapêuticos, redução de crescimento, desperdício de ração e menor qualidade do produto final. A indústria do salmão tem combatido o parasita essencialmente com tratamentos químicos de diversas formas; contudo, nos últimos anos provas de resistência têm sido detectadas o que torna inexequível o abuso de químicos nas jaulas de salmão, aumentando a despesa dos produtores e o impacto negativo no ambiente. Por conseguinte, uma abordagem de custo-eficácia à alternativa dos tratamentos farmacêuticos tornou-se uma prioridade urgente para a indústria de aquacultura. Como resultado surgiram as co-produções com os “peixes-limpadores”. O *Cyclopterus lumpus* é uma espécie recente que tem revelado uma boa eficácia na remoção dos parasitas nas jaulas, para além de que, é uma solução amiga do ambiente e reduz o impacto financeiro. A aquacultura de *C. lumpus* tem sido a maior aposta da Noruega nos últimos cinco anos. No entanto, é uma espécie com pouca informação científica em termos de biologia e condições de produção intensiva.

Este trabalho visa as práticas gerais de produção de aquacultura do peixe-lapa na empresa Nordland Rensefisk AS em Lovund, Noruega. Uma vez que as informações acerca desta espécie são tão escassas, realizou-se um estágio profissional na produção e um ensaio na fase larval. O ensaio teve como objectivo principal de avaliar as diferenças entre duas rações: Skretting e Otohime. Este realizou-se desde a fase de eclosão até ao momento em que os alevins atingiram aproximadamente 1 g de peso médio. Analisaram-se os parâmetros de peso médio, comprimento e taxa de mortalidade. A marca Skretting revelou ser a ração que melhor favorece o crescimento larval no menor espaço de tempo. A fase larvar sendo o período de maior vulnerabilidade denota que a escolha de uma ração adequada marca a diferença em termos de performance de crescimento e o impacto económico da empresa.

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I. Introduction

1. Overview of salmon production in Norway

Salmon, is the common name for several species of fish of the family Salmonidae, however it is more associated to *Salmo salar* (L.) the Atlantic salmon. This fish is consumed worldwide being known as a very healthy ingredient to our regular diet, due to its high content of protein and Omega-3 fatty acids in addition to its appetitive flavor and easy confection (ISFA, 2015; Marine Harvest, 2015).

About 70% of the world's salmon production comes from farming. It can be reared in offshore nets or placed in sheltered waters such as fjords or bays. The whole cycle of production takes around three years (24-40 months) to complete as it is represented in Fig. 1 (GSI, 2015; Marine Harvest, 2015). First, the eggs are fertilized with milt and then incubated in shelves for approximately 30 days (1). In the first year, juveniles grow until they weight approximately 100g in freshwater tanks (2). The fish is then transported to sea cages (3) and remains there for 14-24 months, growing between 4-5 kg (4). Upon reaching harvest size, the salmon is transported to processing units, where it is slaughtered and gutted (5). Following costumer preferences the salmon is usual sold as a whole fish or as a fillet in ice packages (6) (Marine Harvest, 2015).

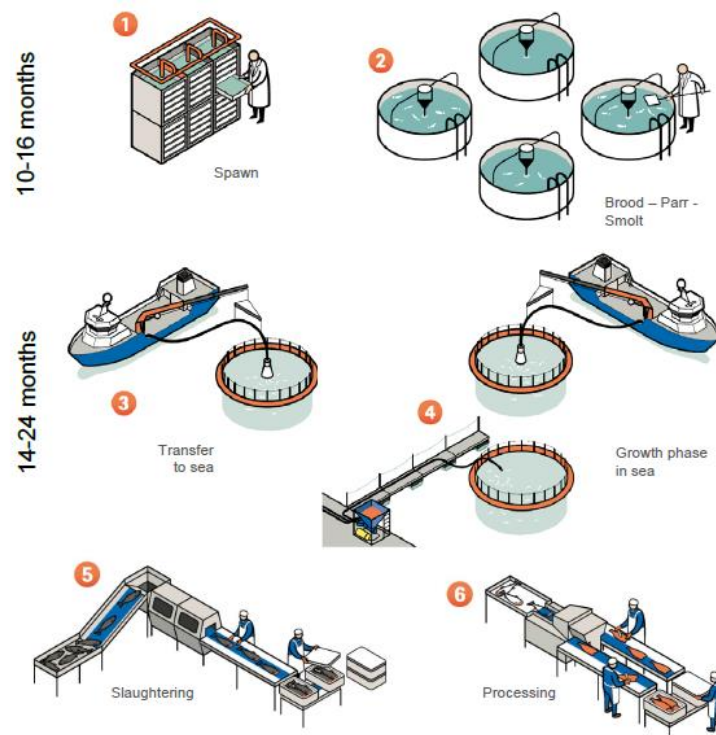


Fig. 1 Farmed salmon cycle, (adopted from Marine Harvest, 2015).

Salmon is traded all over the world and being sold as a fresh product, time and cost of distribution are a high concern in order to choose market targets. Norway is the biggest producer in the world having reached its maximum peak on the last decade with 1 314 584 tons produced in 2015 (Fig. 2). It has 994 seawater production sites involving 160 companies (Norwegian Directorate of Fisheries, 2015).

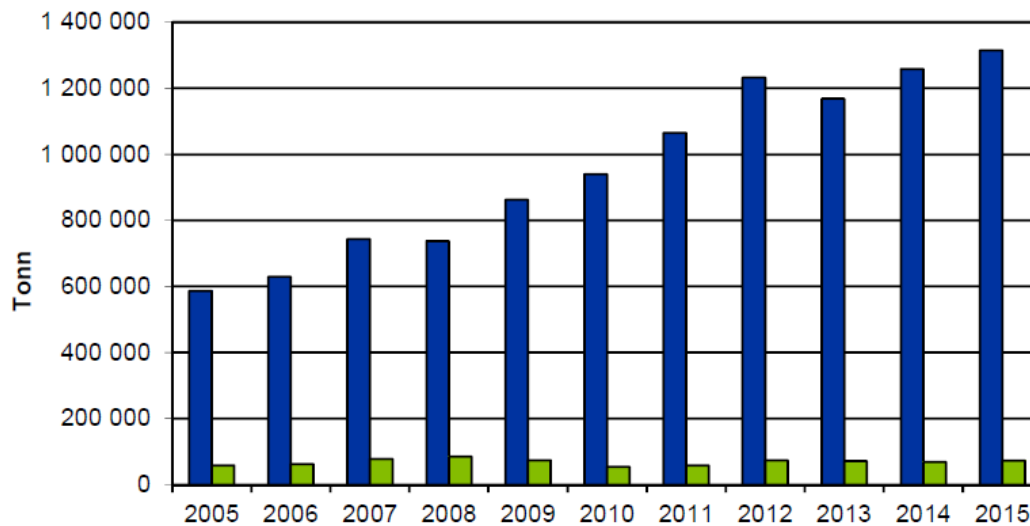


Fig. 2 Sale of Atlantic salmon (blue) and rainbow trout (green) 2004-2015 (Norwegian Directorate of Fisheries, 2015).

This volume of production has been primary due to contribution of the ten biggest salmon companies according to 2014 values:

Table 1 Top 10 Salmon Norwegian Producers (adapted from Marine Harvest, 2015). *All Fig.s in tonnes GWE (gutted weight equivalent) for 2014E.

	Top 10 Norway	Harvest*
1	Marine Harvest	258 000
2	Salmar	141 000
3	Lerøy Seafood	133 000
4	Cermaq	53 000
5	Nordlaks	38 500
6	Nova Sea	38 000
7	Grieg Seafood	37 500
8	Alsaker Fjordbruk	25 500
9	Norway Royal Salmon	22 500
10	Sinkaberg-Hansen	20 500

Marine Harvest is the biggest producer, producing about one quarter of the total volume in Norway. Salmon sales have been growing around 9% a year since the last two decades and the value of salmon has tripled since 2004. Even though the total volumes of production are growing each year there is still a strong demand for the product (Marine Harvest, 2015).

2. Sea lice dilemma

Fish farms are an epicentre of sea lice infestations. The aquaculture industry has been struggling with this matter for many years and fighting it represents a big part of the production cost. It is known to cause reduction in growth and increased mortality rates which influence market prices. Therefore there is an emerging need of prevention and treatment measures (Bjørn *et al.*, 2001; Torrissen *et al.*, 2013; Liu and Bjelland, 2014).

In Norway, in 2014 researchers estimated salmon lice to cost the industry about 350€ million. The cost of slaughtered marked fish went up to $\approx 0,54\text{€}/\text{kg}$ for salmon produced in 2014 due to the huge amount of money spend on lice treatments (Costello, 2009a; Iversen, 2015). Audun, researcher in Nofima institute said “Cost has risen 40% in real values from 2011 to 2014.” It is noticeable on the Fig. 3 the intensification of chemotherapeutics being applied from one year to the other in order to fight sea lice.

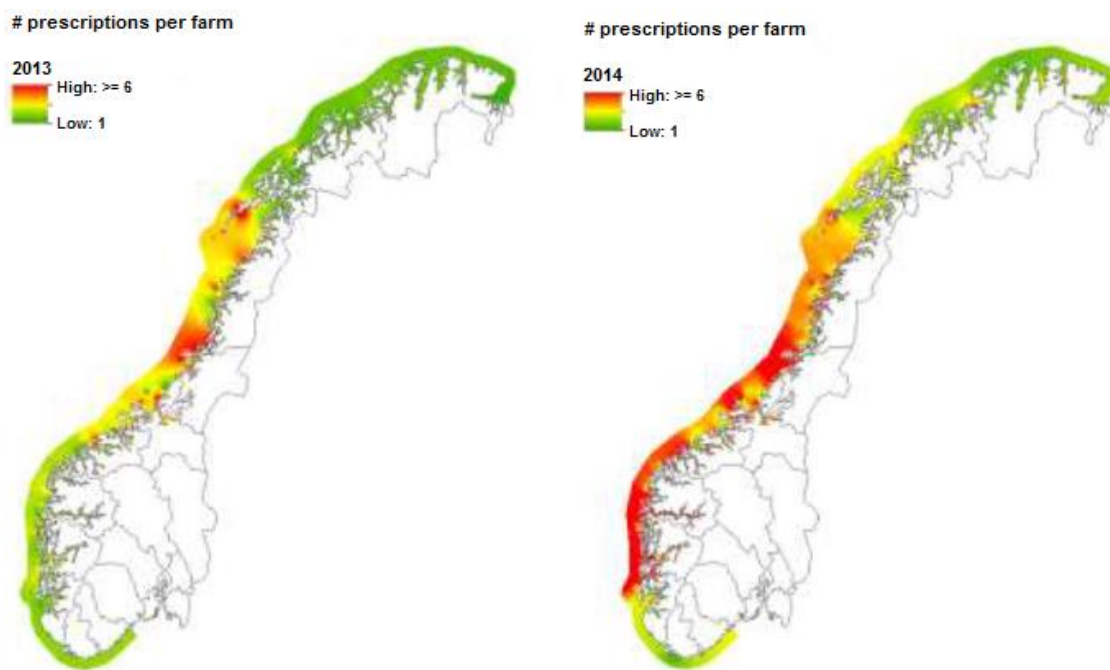


Fig. 3 Number of prescriptions per farm location covering all substances used to control salmon lice. Dark red denote areas where more than 6 prescriptions per location, while dark green denote areas where the expectation of one treatment is approached (Grøntvedt *et al.*, 2016).

2.1. Sea lice

Salmon lice is the most common parasite to the Atlantic salmon, which can be transmitted from farmed to wild salmon, as well as vice-versa. Lately It has been reported that the number of sea lice have been increasing over the last two decades, essentially in salmon pens (Liu and Bjelland, 2014). Fish farms, stocks thousands of fish constituting a perfect breeding ground for lice, therefore intensify the number of lice in surrounding waters. Besides, salmon farms are located in sheltered bays or estuaries coinciding or being close to migratory routes of wild adult/ juvenile salmon submitting wild stocks to be at vulnerable risk (Torrissen et al., 2013). High proliferation of lice can also physically harm any fish, raising the challenge on a level of animal health, welfare and environmental. Aquaculture industry has a major role and interest in retaining lice population as low and controlled as possible, minimizing the impact on salmon and guaranteeing that the louse are still predispose to treatment (FHL, 2011).

Sea lice members of the copepod family Caligidae, *Lepeoththeirus* and *Caligus* are naturally part of salmon and trout environment (Imsland et al., 2014a). *Lepeophtheirus salmonis* (Krøyer, 1837) is the dominant specie found in salmon farms in Northern Europe. The life span of this louse is still in debate among researchers, but it is predicted to be between 25-45 days, according to different temperatures. During spring/summer their reproduction is substantially high, although, it has been reported that adults can survive over-winter on wild salmon (Treasurer, 2002; Hamre et al., 2013). The cycle of this louse (Fig. 4), includes the following stages: two initial larval stages called Nauplius (non-feeding and planktonic); the third stage is copepods stage where the louse attaches itself into the host by a thread ('frontal filament'). At the attachment site, copepods start to turn into 4 sessile chalimus stages. The lice undergo two immature pre-adult stages, where they move freely along the host to feed, until reaching adulthood. The female carries a pair of external egg sacs or "eggstrings" with 500-100 eggs, producing six to eleven broods over her life time. Afterwards, the eggs float on the surface hatching in to the first stage (Whelan, 2010).

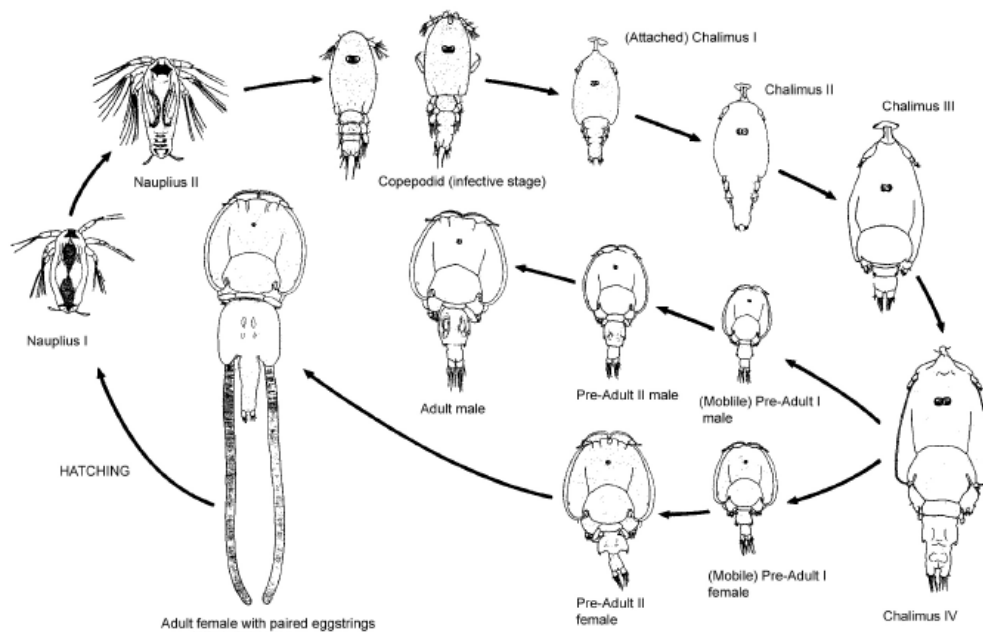


Fig. 4 Life cycle of the sea louse *Lepeophtheirus salmonis* (Whelan, 2010).

Sea lice injure salmon in a diversity of ways. Once it attaches to the host surface and develops to mobile stages, it becomes potentially harmful due to its feeding activity. It grazes the host's skin, causing modifications to mucus biochemistry and discharge, besides causing underlying tissue necrosis. As the damage induced is spreading, it leads to secondary microbial infections (Treasurer, 2002; Costello, 2009b; Whelan, 2010; Torrissen *et al.*, 2013). The fish is liable to suffer: osmoregulatory dysfunction, physiological stress, loss of appetite, declining growth and food conversion efficiency. The host can suffer changes and might cause anemia, reduced lymphocytes and increased cortisol. These are signs of a stressed and weakened animal and depending on the number of lice in the host it can be defined as "diseased".

Sea lice can stick to any part of body of the host, however, they tend to occupy the skin on the head and fins (Fig. 5). If there is a high level of infestation of lice in one area, the feeding activity is also higher which might cause erosion of muscle tissue, fin rays or skull bone exposed (Whelan, 2010).



Fig. 5 Adult female *Lepeophtheirus salmonis* occupying the skin adjacent to the anal fin of an adult two sea-winter Atlantic salmon (~7 kg) (Crawford Revie, 2009).

Norwegian authorities established a new regulation since 2009 for number of lice per treated fish. From January 1st to august 31, 0,5 adult female (one adult female present in every two fish sample) or 3 mobile lice and from September 1st to December 31st, 1 adult female or 5 mobile lice per fish (Liu and Bjelland, 2014).

2.2. Sea lice treatments

In order to avoid costly losses, salmon farmers and research institutes have developed a diversity of methods to prevent and treat sea lice outbreaks. Most salmon farmers use a combination of treatments, depending on the severity of the infestation and the stage.

At the moment, there are multiple ways of treatment/prevention of sea lice, including mechanical measures such as: optical delousing (laser under water), lice skirt (mesh around sea pens), snorkel pen (prevent fish from top layers) among others under development. While medical treatments and cleaner-fish are the most current, prevailing methods are being applied (Langford *et al.*, 2015; Grøntvedt *et al.*, 2016).

2.2.1. Topical treatments

Medical treatments can be applied as bath or in feed treatment. Bath treatment consists of diluting active substances on sea pens, such as: azamethiphos (the only organophosphate licenced for use in Norwegian aquaculture which has been in use since 2008), pyrethroids, hydrogen peroxide (most frequently used) and flubenzuron (Langford *et al.*, 2015; Grøntvedt *et al.*, 2016). Either sea cages are isolated with tarpaulin or fish are transferred to a well-boat, where they are enclosed with a medical agent within a certain time (20-40 min), temperature varies depending on the specification for each substance. This bath treatment is considered topical since the sea lice absorb the

medicine from the water column instead from the host. Emamectin benzoate is incorporated as in-feed treatment.

2.2.2. Cleaner fish

Cleaner-fish present the only sustainable and environment friendly method, in alternative to topic treatments on de-lousing salmonids (Imsland et al., 2014a). Co-production of cleaner-fish and salmonids is a practice which goes as far as the 1970's. It is an alternative to fight the costs of medicine where, more and more, lice are prevailing and resisting to such treatments (Burridge et al., 2010). It has a low impact on the environment and is a reliable lice control method (Bornø and Linaker, 2014; Imsland et al., 2014b). The cleaner-fish is introduced in sea cages, where it acts as lice predator, grazing on salmonids skin and feeding of it. Of equal importance is the challenge to determinate the right amount of cleaner fish per sea cage. Commonly, it fits 5-20% of salmon density, however, must bear in mind the temperature, any presence of disease and the level of infestation of lice (Willumsen, 2001; Imsland et al., 2014a). The number of cleaner-fish within farms has been rising mostly in the last year's, aiming to replace delousing agents, as it is graphically shown below in Fig. 6 (Willumsen, 2001; Burridge et al., 2010).

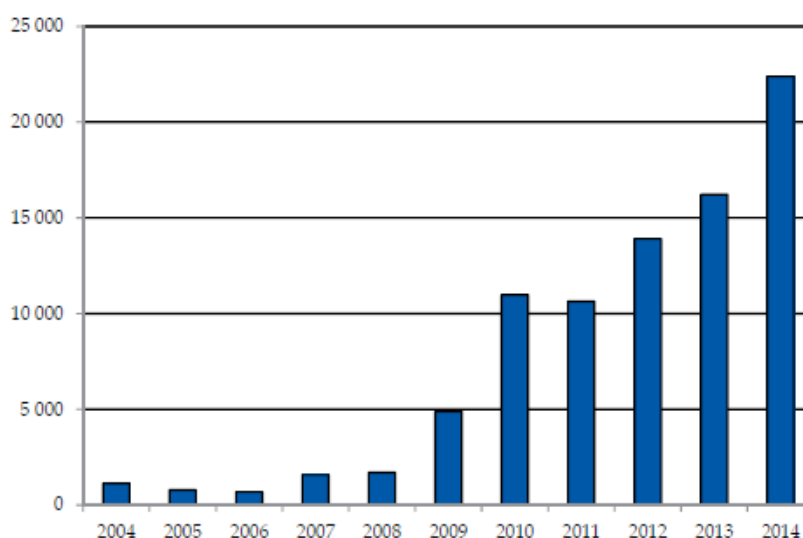


Fig. 6 Number of cleaner fish in the cages with Atlantic salmon and rainbow trout 2004-2014 in Norway (Norwegian Directorate of Fisheries, 2015).

The most frequent species used in Norway in recent years are: the lumpfish (*Cyclopterus lumpus*) and various species from the *Labridae* family, the Wrasse lineage such as: goldsinney wrasse (*Ctenolabrus rupestris*), corksling wrasse (*Symphodus melops*), ballan wrasse (*Labrus bergylta*), rock cook (*Centrolabrus exoletus*) and cuckoo wrasse (*Labrus mixtus*) (Bornø and Linaker, 2014). Ballan wrasse has been farmed several years in constant number whilst the lumpfish production has been striking a high number and

bigger impact over the last three years (Bornø and Linaker, 2014). According to the Norwegian Veterinary Institute the number of ballan wrasse and lumpfish were very similar in 2013, meanwhile the production of the remaining species of wrasse are declining. Nevertheless, not only farmed cleaner-fish are currently being used, most of it is caught in the wild to complement the percentage demanded in each sea cage. In the end of 2015 the total volume of cleaner-fish caught was around 850 tons in a value of \approx 23 million euros (Waatevik, 2016).

It is known that low water temperatures represent a bigger challenge for some cleaner-fish, mainly to wrasse genders. They show loss of appetite for lice when placed in northern waters, limiting their spatial distribution through all Norway (Holst, 1993; Schaer and Vestvik, 2012; Eriksen *et al.*, 2014) as a consequence farmers from the north of Norway experience great difficulty accessing large quantity of them (Willumsen, 2001). Though, wrasse is still produced to combat sea lice, only two companies in the entire country produce it (Waatevik, 2016). Lumpsucker became a new option, being natural northern specie better adapted to cold waters have been proving their efficiency until now (Bornø and Linaker, 2014; Imsland *et al.*, 2014a).

II. Lumpfish

(Cyclopterus lumpus)

1. Lumpfish (*Cyclopterus lumpus*)

Lumpfish is often assumed to be a rocky bottom demersal fish, although it is proved to be semi-pelagic fish, spending their adult life in pelagic zones in open sea (Davenport, 1985; Tún, 2014). *C. lumpus* is widely spread through both sides of the Atlantic Ocean (Fig. 7) showing preference to high latitudes as the North Atlantic where the biggest populations can be found at: Maine, Canada, Greenland, Iceland, Norway and Baltic Sea (Davenport, 1985; FAO, 2006; Schaer and Vestvik, 2012; Eriksen *et al.*, 2014; Tún, 2014). Despite the perception of lumpfish as a cold water fish it can be also found in warmer waters alongside the Portuguese coast and South Galicia (Bañón *et al.*, 2008).

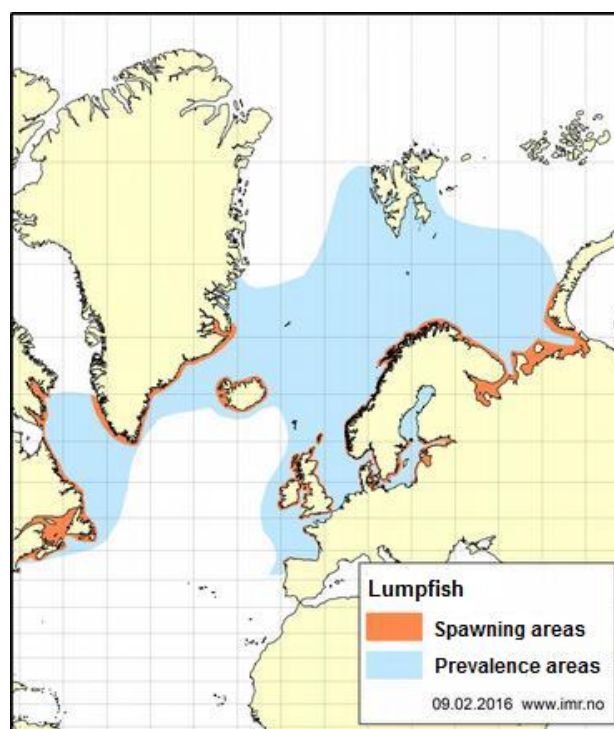


Fig. 7 Geographical distribution of lumpfish (adopted from HaVet- Fiskehelse, 2017).

The lumpfish (*Cyclopterus lumpus* L. 1758) is the only species of the genus *Cyclopterus* by Davenport, 1985. The most common name is lumpfish or lumpsucker, although, it has different names in different countries: rognkjeks (NO), stenbit (SE), poule de mer (FR), peixe-lapa (PT), lumpo jibado (SP), among others. It is usually caught for their roe/caviar market (Davenport, 1985; FAO, 2006).

C. lumpus is highly distinguished by its charismatic form. The head is small with rounded nose. Instead of scales it has a very thick skin with rows of opposed tubercles alongside the whole body. The dorsal fin thickens with age, while the pelvic fins form a ventral disc or a “sucker”, which enable it to adhere to rocky or algae substrate (Davenport, 1985; FAO, 2006; Tún, 2014). The flesh of lumpfish is very loose, the bone density is light and it

does not have a swim bladder, assumed to be an adaptation for pelagic life (Tún, 2014). Lumpfish exhibit a sexual dimorphism in size, skin color and plasma (Mikkelsen, 2013). Females can reach up to 50 cm and are often blue to dark grey color, on the other hand males are between 17-38 cm and display nuptial colors like pink, orange and red (Fig. 8). Juveniles can show different colors depending on their habitat or water temperature, from light green to bluish, grey, brown, and red with spot mixture (Thorsteisson, 1981; Davenport, 1985; FAO, 2006).



Fig. 8 Female (left) and male (right) lumpfish (adopted from Wenneck, 2005).

Females can reach sexual maturity around 5-6 years of age while it takes only 4-5 years for males (Albert *et al.*, 2002). Spawning season occurs frequently early spring February-May however, it has been reported it can last until middle of summer and beginnings of autumn (Schaer and Vestvik, 2012; Pampoulie *et al.*, 2014 ; Tún, 2014).

During breeding season, males reside in shallow waters establishing their territories and nest sites. Males remain in the nest site protecting and ventilating the eggs around 190-220 degree-days until hatching. Females stay in nearby coastal waters about 3-4 weeks where they lay two to four batches of eggs, containing from 80 000 to 200 000 eggs per spawning season. Males can fertilize eggs from different females in a single nest while females return into deep sea after spawning (Tún, 2014). The eggs are usually around 2 mm in diameter and can display different colors per batch, such as pink, orange, purple, yellow, green or blue (Thorsteisson, 1981; Davenport, 1985;). The eggs develop an adhesive propriety in order to stick to the substrate and remain all bounded together until hatching (Benfey and Methven, 1986). Once the larvae hatch, they measure about 4- 7,4 mm and possess a yolk sack for 15 days, as well as the adhesive disc is already functional (Benfey and Methven, 1986; Schaer and Vestvik, 2012). Within the first week larvae start to feed on small and sessile preys (Mitamura *et al.*, 2012). During early juvenile phase they reside in the intertidal pool, more commonly found in algae areas, especially *Laminaria* or eelgrass, providing shelter, camouflage and plankton as a food source. On the first summer/autumn, juveniles swim towards the open sea within a range

of 50-300 m depth, feeding on a diversity of zooplankton (e.g. krill, copepods) jellyfish and polychaetes (Moring, 2001; Mitamura *et al.*, 2012; Schaer and Vestvik, 2012).

2. Lumpfish Aquaculture

C. lumpus was frequently found in salmon nets, then, in 1998, a salmon farmer decided to dissect a lumpfish. Surprisingly, he found 160 lice inside the stomach as we can observe on the example below (Fig. 9) (Willumsen, 2001). After several trials, lumpfish turned out to be a successful tool to combat lice, plus their wide natural range in northern waters became to be decisive in order to attempt lumpfish production in large scale (Schaer and Vestvik, 2012).



Fig. 9 Lumpfish which ingested around 100 lice (Jonassen, 2016).

Lumpfish production started being more successful and showing positive results in salmon cages, a number of fish farms slowly changed their production from cod to lumpfish. By the end of 2015, there were 30 lumpfish farms either starting or active spread over all Norway, besides 6 more new prospects planned to initiate in 2016 (Norsk Fiskenaering, 2016; Waatevik, 2016). Lumpfish aquaculture is increasing in order to satisfy the need of salmon free of lice. Even though between 15 to 22 million lumpfish are expected to be introduced in sea cages by 2017, it is still will not be enough (Davidsen, 2015). Considering all the production of cleaner-fish up until now there is still a great gap between supply and demand (Fig. 10), however it is estimated that within 5 years the demand will be met (Waatevik, 2016).

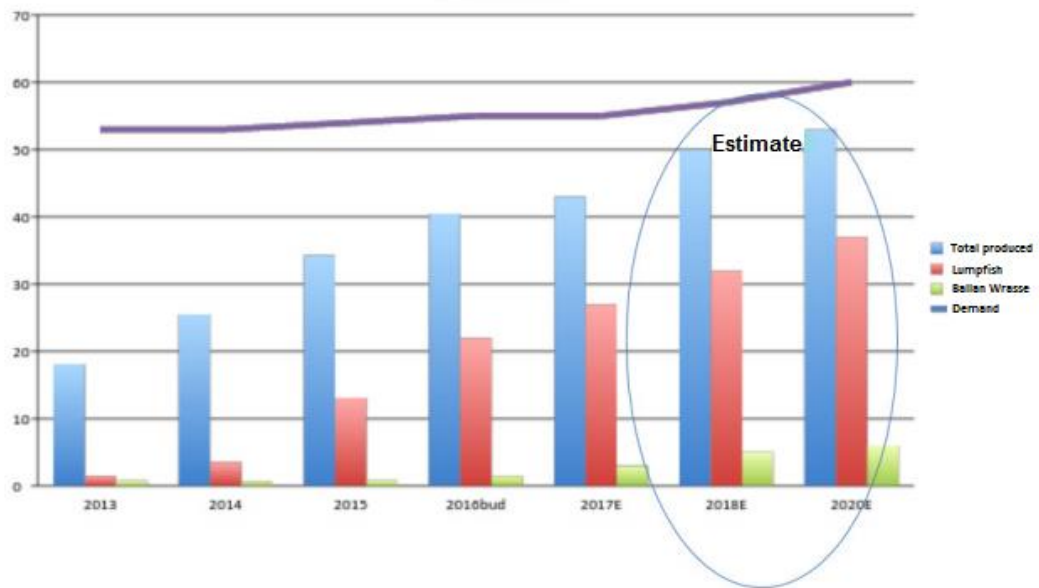


Fig. 10 Relation of demand and cleaner-fish estimations (adapted from Waatevik , 2016).

III. Internship in Nordland Rensefisk AS

1. Nordland Rensefisk AS

Nordland Rensefisk AS (NR) is located in Lovund (Fig. 12), a small island situated in the north part of Norway, on the edge of Polar Arctic Circle.

At the beginning, Nordland Rensefisk AS was built as a cod farm in 2009. In 2010 the first attempt at farming cleaner fish was made, using wrasse, goldsinny and later on 2012 with lumpfish. The lumpfish experience had such good results that since 2013 it was decided to only focus on lumpfish production (Davidsen, 2015; Lei and Jonassen, 2016). In 2015 the company sold fish in value of 30 million NOK (Norwegian krone) (≈ 3.2 € million) representing 1,5 million fish (Fig. 12). Due to current progress in 2016, NR initiated to expand their facilities in order to double the amount of production, with the purpose to fulfill the request of their clients (Ulvan, 2016).

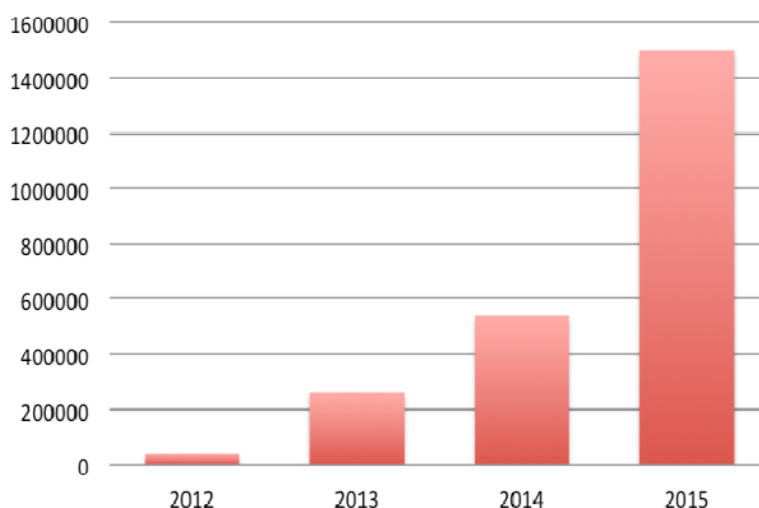


Fig. 11 Production of lumpfish by Nordland Rensefisk AS (adopted from Ulvan, 2016).

N. Rensefisk is directed by Lars Jørgen Ulvan since 2013 and owned by Nova Sea (20%), Marine Harvest (20%), Sinkaberghansen (20%), Midt-Norsk Havbruk (20%), Bjørøya Fiskeoppdrett (10%) and SalmoNor (10%) which is responsible to provide the same percentage of fish to each company (Davidsen, 2015).

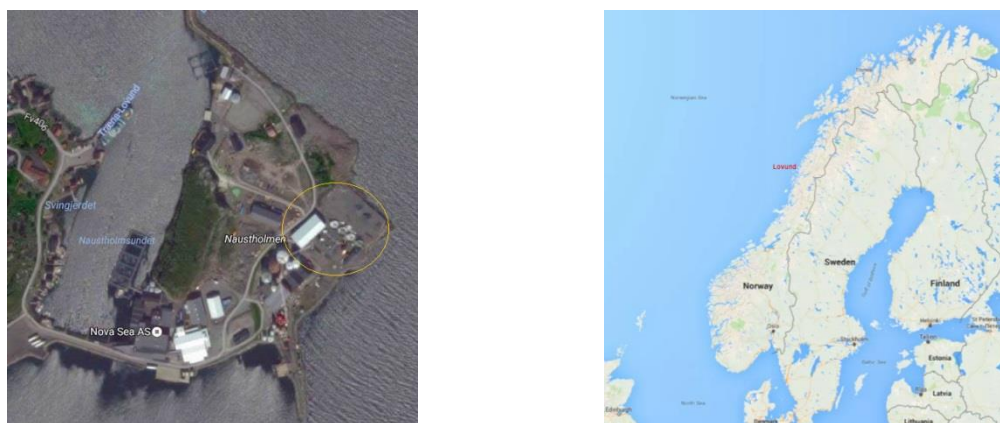


Fig. 12 Lovund at the right picture and Nordalnd Rensefisk AS at the left picture (Simpleview, 2017).

The company working hours start at 8:00 am and finish at 16:00 pm. The first half an hour is used to discuss all goals needed to be executed during the week and throughout the day. The first person who arrives should take a tour through all departments to check if everything is running as normal. After the first four hours of work it is time for lunch break at midday, for thirty minutes, following by the four hours of work left. In the end of the day all data is registered. Each employee takes night rounds for every day during one week to check all technical and fish departments. The weekends are rotated by the workers carrying out daily and essential routines.

2. Facilities and husbandry

2.1. Broodstock

Broodstock is caught in the wild by a local fisherman. Fishing starts from February until May restarting from September until November. Even though, it is reported by several authors that the spawning season is between April and May (Goulet, 1985; Baird and Stevenson, 1988; Goulet and Green, 1988; FAO, 2006; Mitamura *et al.*, 2012; Kasper *et al.*, 2014; Tún, 2014; Marine Research Institute, 2015), more and more farmers are observing that the spawning activity actually has wider range (Schaer and Vestvik, 2012), however, a special license is required to fish out of official schedule. The fishing process consists of placing nets from 5 to 25 m deep in shallow waters during the night. As males and females have a different size, the meshes of the nets are adapted for each sex, not compromising other species.

Wild fish caught are held in outdoors tanks of 6 m³ and separated by sex. The fish is held until all females are striped. Females are checked 2-3 times a week to ensure there are no eggs releases inside the tanks, since once it happens it can trigger other females to do so. It is important to spot the right time of spawning to avoid a premature batch since the

egg's final maturation occurs within the last hours prior to the spawn. It is an important process because oocytes take up a lot of water from the egg liquid during this period, and a change in the ionic composition of eggs during this process also occurs (FHF, 2016). A female can reach up to one-third of its own weight in eggs, although 50,000 eggs or 0,5 L are a more common value per batch (Schaer and Vestvik, 2012). The eggs are surrounded by an ovarian fluid which always surpasses the eggs volume. It is believed that it acts as a spawning barrier to prevent reflux of seawater into the oviduct, avoiding the egg batch to harden and clog inside (Davenport, 1985).

2.1.1. Stripping

There are some recommendations to be aware when it comes to stripping roe from Lumpfish. The Fig. 13 demonstrates some of the steps (A to F) taken and before any procedure is performed all fish are anesthetized.



Fig. 13 Stripping procedure (FHF, 2016).

- A- As mentioned above, is important to select a “ready” female and one of the differences can be seen from picture (a) to (b).

- B- The female should be swollen and can occasionally display a reddish gonadal pore. The use of disposable gloves is obligatory in order to avoid damage to the skin of the fish and to minimize the level of contamination.
- C- The gonadal pore is wiped with towels to prevent contact between sea water and roe.
- D- It might be needed to penetrate gently the gonadal pore to remove the obstruction of the membranes that maintain it closed.
- E- Some pressure is induced along the abdominal captivity up to the gonadal pore and then it is squeezed moderately. If the fish is ready the roe should come out easily.
- F- In the end, most of the eggs are removed and the fish will be empty inside.

At the moment of stripping, the eggs are poured into 1 L cup (Fig. 14) where the total amount is registered, usually between 200-500 ml per batch.



Fig. 14 Eggs after stripping in Nordland Rensefisk AS.

Male's milt should be ready to use. Concerning to the origin of milt, it is used pure milt or defrosted milt in NR. The milt is directly harvested from the male, done by just squeezing the abdominal captivity which withdraws some drops (6-8) and are placed into each batch (Fig. 15). In NR at least 2 to 3 males are used, just in case one of them happens to be infertile.



Fig. 15 Addition of sperm to egg batch's in Nordland Rensefisk AS.

Unfortunately not all males provide flowing milt, so it is often needed to dissect the fish for the gonads to be removed and either stored in the freezer or cryopreserved. It has the advantage to be storage and it is always desirable to access sperm without killing so many animals. The gonadal extraction process consists of: dissecting the whole gonadal organ (a); grinding the gonads (b); screening the milt (c); transferring it to a cell culture bottle (d) (Fig. 16).

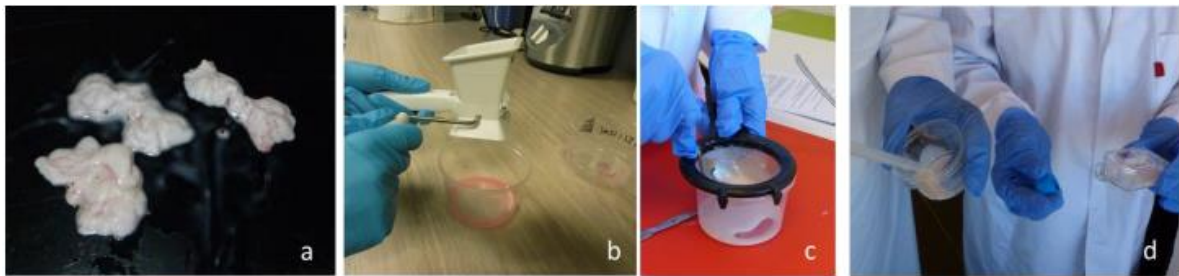


Fig. 16 Method to extract milt to defrost or cryopreservation (FHF, 2016).

Before the milt is inserted in the bottle, it should be diluted with a physiological solution (i.e. AquaBoost® SpermCoat from Cryogenetics) which allows the sperm to have better access to oxygen during storage. In the case of being stored in the fridge it is important to be aware to shake them gently 2-3 times a day to ensure all the sperm gets oxygen. Another option is cryopreservation, by freezing the sperm with nitrogen it allows it to be stored for at least over one season. Under a project executed in Norway by Nofima it was shown that using frozen milt and cryopreserved milt gave over a 90% fertilization rate, respectively 92.3 and 91.2%. While fresh milt was proven to have >96% fertilization rate in all experiments (FHF, 2016).

2.1.2. Fertilization

Dry fertilization is the most common way to fertilize the eggs, which consists of mixing male gonads (milt) among the eggs, where it stays for 5-10 min, before sea water is added. Sea water should cover at least the amount of eggs, translating into more or less the same volume as the eggs volume. When sea water is added it enables the egg to swell up and close the sperm entrance (microphylen), only then the fertilization process begins (FHF, 2016). In contact with sea water, the eggs gain a sticky characteristic and get harden, forming an egg mass with the shape of a cake as it can be seen on the Fig. 17 (Lei and Jonassen, 2016). After that the eggs are transported to the incubators.



Fig. 17 Lumpfish roe in incubators (adopted from ACFFA, 2015).

2.2. Incubation

Nordland Rensefisk has 38 incubators as illustrated above from Sterner Fish Tech, with a maximum capacity of 1,5 L. Usually each incubator holds only one batch, but there are cases where smaller batches of around 250mL can be combined in a single incubator. Incubation period depends on the temperature which it is lead on. In the natural environment they occur most of the times at 5 °C and take approximately 40 days to hatch (Davenport, 1985; Schaer and Vestvik, 2012). In a project ran by Akvaplan-niva AS, results showed the values to be more precisely are 255-353 day-degree at 5° C and 268-386 degree-day at 10°C. In NR, when the temperatures start to fall, a heating pump is used to maintain the temperature between 8-10 °C. At 10° C the eggs take around 28 days to hatch, 270 degree-day, while in summer can reach 12-13° C maximum.

During the whole process the eggs are not touched. They remain in a very dim light 20 lux area with a gentle flow induced in each incubator. The eggs have the temperature checked daily in order to have a better prediction of the hatching time. The oxygen is kept

always above 90%. Normal survival rate at hatching is around 70% depending on the fertilization rate. The size of the eggs is approximately 2.0-2.5 mm in diameter during whole process (Davenport, 1985; Nytrø *et al.*, 2014; FHF, 2016).

2.3. Larval Rearing

The eggs are then transported to the start-feeding department 2-3 days before they hatch. The start-feeding consists of 9 green tanks of 1 m³. Usually each tank receives eggs from two different incubators unless there is not enough room, in that cases a maximum of 4 incubators are inserted per tank. One tank can hold around 100,000-150,000 larvae. The flow starts at 5 L/min increasing up to 15 L/min throughout the whole process, where it is adjusted according to their needs and density. The oxygen must always to be kept above 80% saturation. The photoperiod is continuous (LD 24:0) and kept dim for the whole process. The tanks are equipped with an automatic feeder which provides drops of food 24h a day. The food used on this stage is administered from the third day post-hatch with Skretting Gemma Micro 150, on the following 2-3 weeks it is gradually combined with Skretting Gemma Micro 300; according to the density presented it can be given up to 5% of biomass every day. The larvae stay in these tanks for 6-8 weeks where it reaches 0,05 g. This period is when they are most vulnerable to pollution and bacterial threats inside the tank, which demands laborious cleaning to avoid an oil layer being formed on the walls from the feed and bacterial strands. When the cleaning is made, all feed excess is flushed away and all dead larvae are “vacuumed” and counted daily to control the mortality rate. If there are free tanks available after one month, larvae are moved to a new washed tank to ensure the good environment conditions last longer. If there are not, two tanks are merged into a new tank in the next section of growth.

The newly hatched larvae measure around 4,5-7,4 mm in length and weight approximately 2,4 mg. They possess a functional suction disc and a small yolk sac which lasts for a few days (Davenport, 1985; Schaer and Vestvik, 2012). The first behavior right after hatching is to stick to the wall of the tank where they remain most of the time as is seen on the Fig. 18.

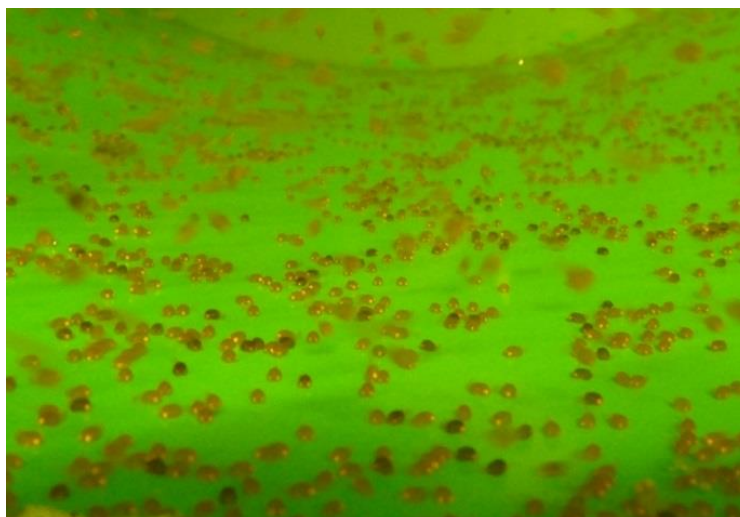


Fig. 18 Lumpfish larvae stick to the wall after hatching in Nordland Rensefisk AS.

2.4. Early Juvenile Hall

Juvenile phase hall holds 33 green tanks, which are disposed in 3 distinguished areas (Fig. 19). The tanks are equipped with an automatic feeder for 24h and continuous light, between 30-70 lux. The oxygen is maintained at minimum 80% where normal water exchange is 1,5 times per hour.

- Section A
 - i. 7 tanks, 1,8 m³ capacity, 2 mm of outlet;
 - ii. Feeding: Skretting Gemma Micro 300 plus, Gemma Wean Diamond 0,5;
 - iii. Fish size: from larval rearing 0,05 g to ≈1 g, up to 4 months old.
- Section B
 - i. 10 tanks, 2,5 m³, 3mm of outlet;
 - ii. Feeding: Skretting Gemma Diamond 0,5/0,8;
 - iii. Fish size: 1 g to ≈5 g, up to 6 months old.
- Section C
 - i. 16 tanks, 2,5 m³, 5mm of outlet;
 - ii. Feeding: Skretting Gemma Diamond 0,8/1,0/1,2;
 - iii. Fish size: 5 to ≈10 g, up to 8 months old.



Fig. 19 Early juvenile hall in Nordland Rensefisk AS.

Fish survival depends on adequate feeding, good water quality and proper ratio space/biomass, avoiding illness or injury. To achieve these parameters handling fish is necessary, all handling is done in a controlled way and preferably without taking fish for a long time out of water (Moksness *et al.*, 2004; Melbu Systems, 2013). Fish wellbeing is taken into high account in NR and all workers are aware of its importance. Daily and periodic measures are implemented as to ensure the health and welfare of the fish throughout the whole cycle of production so that fish can express their natural behavior as much as possible. Daily routines are performed in all tanks consisting of: checking oxygen and flow levels; flushing the tank to get rid of feed debris; removal of dead fish and feeding. Periodic measures are equally important demanding a higher scale of labor. In this department, average weight measures are taken weekly, grading is done upon tanks evaluation and dip vaccination is completed. Dip vaccination is usually performed when fish reach 1 g at the least coinciding with the first/second grading which can be executed simultaneously. The aim of vaccination is to boost the immune system against vibrio strains that are proponent to occur later in the production, such as: *Vibrio anguillarum*, *Vibrio logei* and *Vibrio wodanis*. At this point, fish (0,5 to 1,0 g) might show signs of hassle (tail biting) to each other, which provides a fertile ground for bacterial infections. The most effective way to prevent that is to maintain optimal growth. As there are variable growth rates in the same tank, it is necessary to create homogeneous group sizes by grading frequently. It will allow better feeding adjustment, subsequently, reducing aggression and stress (Moksness *et al.*, 2004).

Grading methods are done manually in NR, utilizing grids and with the aid of a pump, nevertheless in a near future all of the process will be done automatically with a grading

machine. It is important to develop approaches which make management as fast as possible, to ease the impact on fish and workers. It is known that fish experience short-term stress, the brief stress caused by grading does not cause serious harm, only long and prevailing stress can lead to decreased immunity, illness and death (Melbu Systems, 2013). After grading, fish do not show any abnormal sign, they start to spread evenly through the walls, swimming activity is verified and after a few hours appetite is seen. Average weight measures are taken every Monday and in every tank, to evaluate how much have they have grown as also to determine the amount of biomass on each tank. Using this data, plus the temperature, measurement and the right feed conversion rate (FCR), feed is adjusted for the rest of the week.

2.5. Juvenile Hall

Juveniles pass from early juvenile hall to the juvenile hall, after their last grading, this transition is executed by pumping the fish from one tank from early juvenile hall to a specific tank in the juvenile hall. This hall has 12 dark green tanks with 30 m³ capacity (Fig. 20). The hall has continuous light comprehended between 40-80 lux, except when fish are prepared to be sent to sea cages or vaccination period where fish are fasted and the light comes down to 10-30lux to avoid stress. Regarding feeding, the tanks have a 24h automatic feeder and Skretting Gemma Diamond 1,2/1,5/1,8 is also given, depending on fish size. High water exchange is required to assure the oxygen level is above 80%, having a maximum exchange rate once per hour. This kind of tanks can hold a high biomass level, up to 30 kg per m³, reaching up to a ton in a single tank. Fish behavior is affected by the biomass inside the tank and feeding, they either reside in schools or resting at the bottom and walls of the tank.



Fig. 20 Juvenile hall tanks in Nordland Rensefisk AS.

From the moment fish are moved to juvenile hall they usually remain there until they reach 8-10 months old, although when growth rate is higher due to temperature, fish can be ready for delivery just after 5,5 months, upon reaching 10 g. Depending on client's request (which can be determined by their sea cages mesh size, fish size or infection incidence), NR usually prepares fish to delivery in two group sizes: 10-20 g or 25-40 g. The delivery can be conducted by two ways, by boat or by truck. The system for moving fish is equal for both kinds of transportation, fish are pumped from the tank and led to a counter in order to have a precise number of fish that are introduced in each compartment. The mean of transport is specially designed for fish transportation, holding fish in proper conditions until final the destination is reached.

In this department, vaccination is executed shortly after their arrival, being performed on fish from 8-10 g. To prepare vaccination fish are fasted and lights are dimmed for two days, this allows them to remain calm and empty their stomach. The solution injected is constituted by strains of *Aeromonas salmonicida* and *Vibrio anguillarum*. The vaccination is performed by an external team where they can inject up to 4000 fish per hour. Each fish is injected with 0,05 ml and the injection point should be between the edge of the suction disc and the gonadal pore like it appears on the Fig. 21 (Pharmaq, 2016).

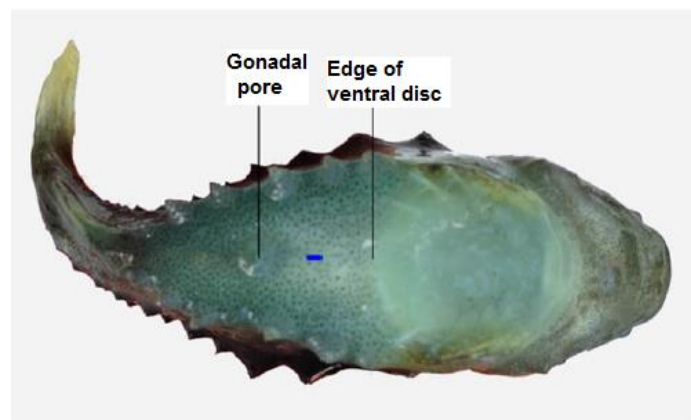


Fig. 21 Right spot of injection in lumpfish (adapted from Pharmaq, 2016).

Currently, there are no clear guidelines on how to use sedation on lumpfish. Unlike salmon, lumpsucker remain tranquil without anesthesia during the whole procedure, plus, it shows a normal behavior in the end. Companies are choosing not to drug fish once it seems to provide them better welfare (Pharmaq, 2016). After three weeks, a veterinary collects a number of fish to analyze the adhesion of the vaccine. Consequently, fish are ready to be delivered after spending 4-5 weeks or 250 degree-day after vaccination.

Apart from vaccination, every month a veterinary visits NR to perform a health check. The Norwegian Food Safety Authority (NFSA) demand a minimum 12 health- related checks

per year which are done by an authorized veterinarian or a fish health biologist. At every visit, the veterinary examine a representable quantity of dead and sick fish. If necessary, fish samples can be taken and they are generally used for histology, bacteriology and PCR examinations. The usual health problems that have been found at this facility are skin- and fins- wounds and lesions. The main disease agents are very often different *Vibrio* species or the *Tenacibaculum* bacteria. In general, such problems can be avoided/reduced by optimizing the environmental living conditions (proper fish density and sorting, correct feeding, clean water). Other health problems, that can be a serious threat for the fish especially after delivery to the sea, are the bacteria-related diseases A typical furunculosis and Pasteurelloses. At NR the vet also takes part in the hygiene and disinfection control of the facility as well as in the control of the vaccination routines and evaluation of the immune reactions of the fish overtime. The samples are sent to Norwegian Veterinary Institute whereas is received a report with diagnosis.

2.6. Technical Department

In NR all technical systems and water circulation blueprints were originally designed for cod farming. Since lumpfish is a marine species, breeding characteristics are similar, only having suffered small changes throughout the time for a better performance. The scheme below demonstrates how water circulation is displayed and how it is treated in a brief description (Fig. 22).

An Inlet Pipe is placed 500 m long and 40 m deep, for water captation. As it enters the Pump Station, the water goes through 2 Pumps of 10.000 L/h and by a filter of 200 µm. The water is led to the first station inside the facility, the Pumps Room. Inside this room, water goes through 2 Inlet Pumps and trough two units of UV. Posteriorly, the water is pumped to the Heating Room which has 3 Effluent Exchangers and a Heating Pump. First, all water goes through the effluent exchangers, this equipment is used to transfer the heat from the dirty water (orange line) coming from the tanks to the cold sea water (blue line), which can raise the temperature up to 1 °C. Still in the same room, the water is divided in two lines: red line, water which goes into the Heating Pump while the blue line goes directly to the Cold Water Tank (in Mezzanine) without any heating treatment. The heating pump warms up the water to any temperature chosen.

Leaving the Heating Room, the two different water channels meet in Mezzanine. In this room, the blue line goes into the Cold Water Tank which has a 10.000 L capacity while the red line goes into the Warm Water Tank with 6.000 L of capacity. Each tank is equipped with a vacuum degasser.

Afterwards, the blue line goes straight to the Juvenile Hall without being pumped while the red line returns to the Pumps Room where it is divided into: Early Juvenile Pump (1) or the Start-feeding/Incubators Pump (2). From Pump (1), the water goes directly to the Early Juvenile Hall, cold water can also be supplied if the temperature suits. To supply the Start-feeding Hall and Incubators, the water goes through the Pump (2) plus a 5 µm filter.

Next, the blue line goes straight to Juvenile Hall without being pumped while the red line returns to the Pumps Room where it is divided into: Early Juvenile Pump (1) or the Start-feeding/Incubators Pump (2). From (1) Pump, the water goes directly to Early Juvenile Hall, cold water can also be supplied if the temperature suits. To supply the Start-feeding Hall and Incubators, the water goes through the (2) Pump plus a 5 µm filter.

When the water is discharged from the tanks, the dirty water (orange line) follows two different ways. From Juvenile Hall the water does not suffer any special treatment and goes directly to the Dirty Water Compartment to be released into the sea. Whereas, all water from Incubators, Start-feeding Hall and Early Juvenile Hall, goes first into a 60 µm drum filter to remove all solid particles. With the aid of 2 Outlet Pumps the dirty water is lead back to the Effluent Exchangers to join the Dirty Water Compartment. At last, all dirty water is discharged into open sea.

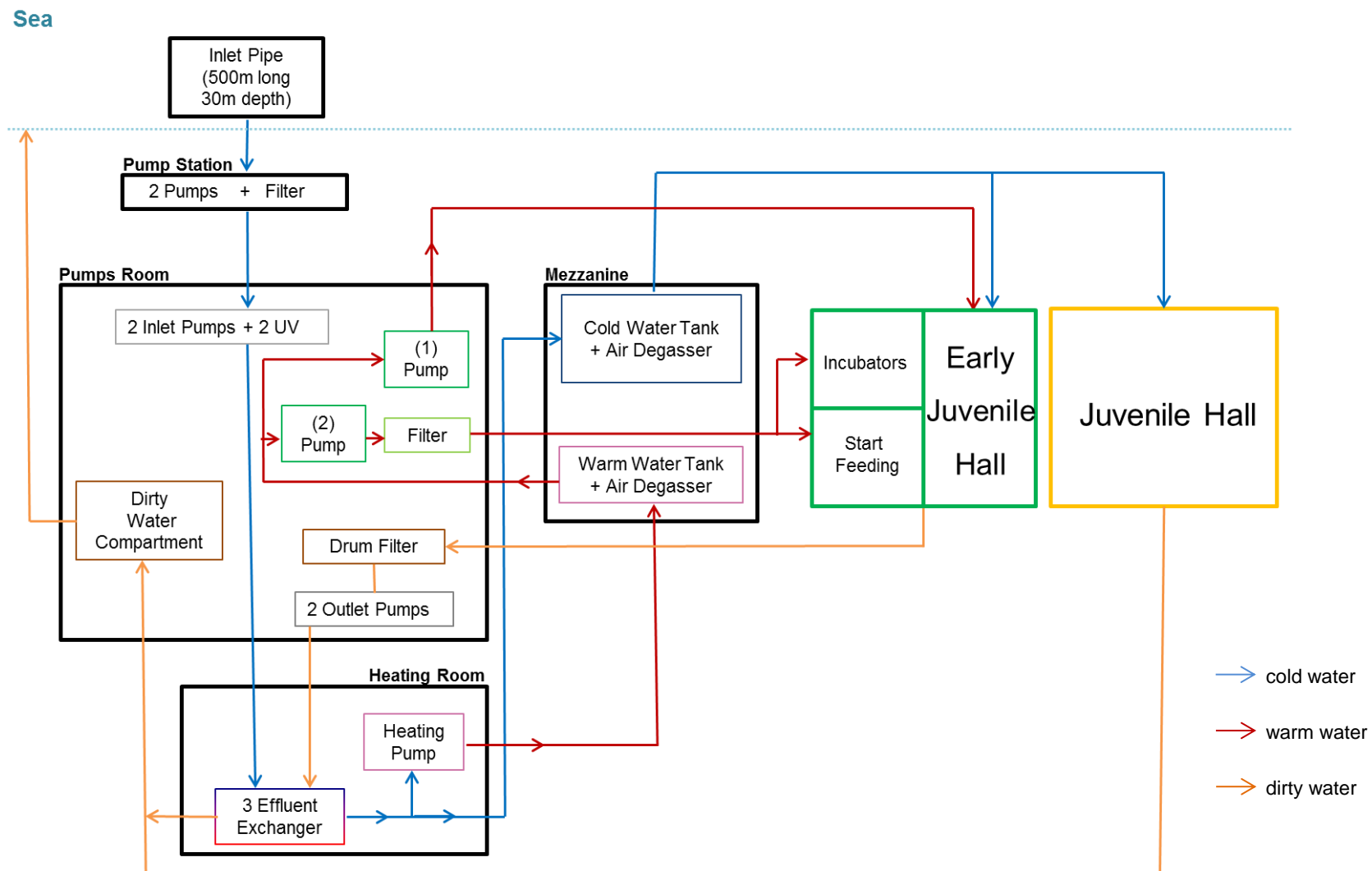


Fig. 22 Water circulation in Nordland Rensefisk AS.

3. Daily responsibilities at Nordland Rensefisk AS

The internship provided by the company has the goal to educate someone as much as possible in order for them to turn into a flexible and professional worker in any department whenever needed. Besides of all daily/periodic routines mentioned above, during the internship, the company has developed a new larval department which provided new responsibilities. As stated, the company is under expansion and renovation for extra capacity. In 2016 the hatching section was relocated into a new hall (Fig. 23) containing 20 larval tanks and a live feed area. As the new hatching hall embraces so many tanks, it might be necessary for a person to take care of it during the whole day. After the feeding trial I was selected to focus and increase my knowledge regarding the larval phase.

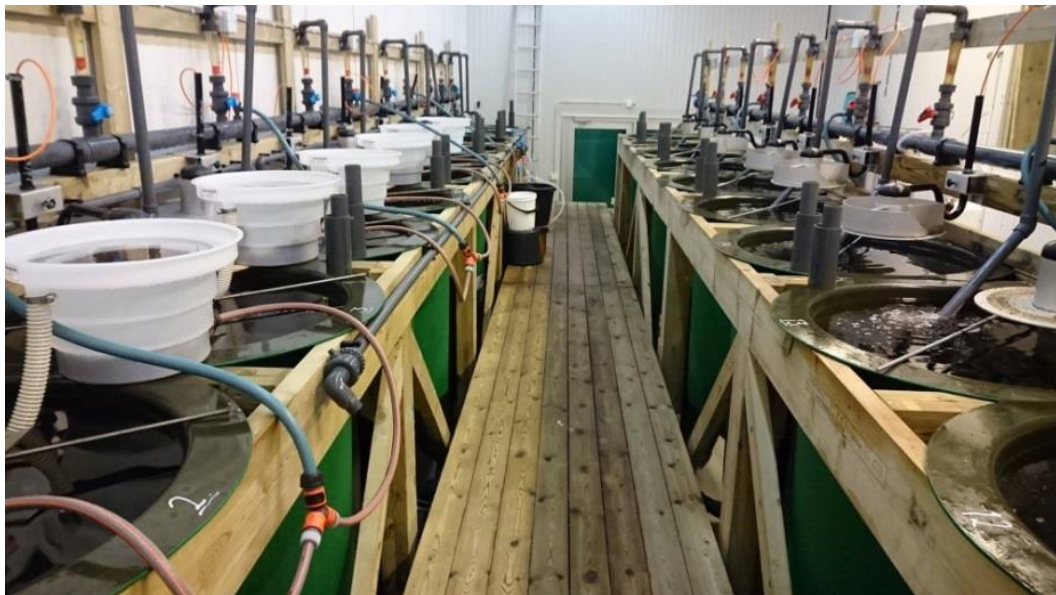


Fig. 23 Larvae hall of Nordland Rensefisk AS.

The biggest difference from the previous larval room is the feeding system which influences the time required to clean and set the feed. The larval phase is divided into two distinct periods: the first segment takes place right after hatching lasting 15 days, where live feed is given and the second period lasting two months where they are fed with dry feed only.

Regarding to daily routine, the first step taken is always to verify how the tanks look in the morning. It is a very important action for feed adjustment. Oxygen measurements and flow evaluation are performed followed by the cleaning of the tanks. During the first days, live feed is administered which is easier and cleaner than dry feed, it might turn out to be a very demanding chore, taking in average 15 min per tank. As cleaning is performed all the mortality is registered and all equipment is disinfected for each tank with a potassium peroxymonosulfate solution.

Nordland Rensefisk AS has been involved in several copepods projects. The results obtained from Planktonic AS were highly successful regarding to: water environment, almost inexistence of mortality, enhancing larvae growth all along the cycle, very easy storage and handling. Planktonic AS is a Norwegian company which has developed a unique cryopreservation technology enabling preservations and revival of live crustacean nauplii. After the project Nordland Rensesefisk AS decided to implement Planktonic AS diet in larvae rearing. The whole preparation takes around 45 minutes and is always arranged on the day before it is administered.

First of all, bags of frozen nauplii are stored in liquid nitrogen bottles. Special equipment is required for their removal. After breaking the bags, the copepods undergo defrosting for a few minutes. With a help of an electric tambour all copepods are filtered as best as it is possible. Finally the copepods mass is inserted into a holding tank of 250 L which requires a water temperature under 5 °C to provide the best revival conditions, on the next day the copepods are pumped to each tank (Fig. 24). Every day the density of nauplii is checked in every tank. This procedure is performed until 15 dph (days post hatch), a transition is performed from live feed to dry feed for a few days until it is merely dry fed.

Regarding dry feed, it is very important to maintain a good environment to avoid bacterial appearances inside the tanks as well as to determinate the right amount of feed for the appropriate density and stage of growth, which might differ from tank to tank. At the moment larvae reach 30 dph, the larvae are old enough to be transferred to early juvenile hall and continue their growth.



Fig. 24 Holding tank of copepods (left) and copepods by magnifier 30x (right) in Nordland Rensefisk.

IV. Evaluation of larval development with two commercial feeds

1. Aim of this study

The larval phase is a crucial period in the ontogeny of the fish, where symbolic changes occur in the structure, physiology, size and morphology. All of these parameters are affected by the first weaning diet (RCN, 2009). Defining the best diet is a bottleneck in most marine hatcheries. Live feed is included on a major part of the marine species as a way to induce live prey motion (rotifers, artemia, copepods) as well their nutritional components (Kolkovski, 2008; Yúfera, 2011; Southgate, 2012). Until the last decade, compound diet was only included/ replaced weeks after hatching. Nowadays, artificial diets have been proof to be successful in a few species such as: European sea bass (*Dicentrarchus labrax*), sea bream (*Sparus aurata*), lumpfish (*Cyclopterus lumpus*), among others (Cahu and Infante, 2001).

Live feed carries a high cost and is laborious to maintain the good quality of the organisms, which is a product more liable to variations of quality and fluctuations of market availability. On the other hand, dry feed has been more and more accepted amongst farmers as a way of simplifying procedures to storage, to handle, plus, its enrichments components are more consistent throughout time (Moksness *et al.*, 2004). The development of formulated diet must fulfil essential nutritional requirements for amino and fatty acids, vitamins, minerals and macronutrients (protein, lipid, carbohydrate) to ensure proper development and high survival rates during the early life stages (Leaver *et al.*, 2008; RCN, 2009).

Since Nordland Rensefisk started to produce lumpfish, it has always been using two different brands of dry feed: Skretting and Otohime. Considering the information mentioned above, the main aim of this study is to evaluate which standard feed provides better larval performance in growth and survival until it reaches 1 g. In that order, data was collected by the following factors: weight, standard length and mortality.

2. Materials and methods

2.1. Experimental design

The experiment started on the 29th of June 2015 until the 10th of November 2015. It was conducted in start-feeding and early juvenile hall of NR embracing two growth phases: larvae and early juvenile phase. Larvae phase starts at day zero until the sixth week, while early juvenile phase goes from week six until the eighteenth. The phases are distinguished on the moment the fish are transferred from start-feeding to early juvenile

hall department. Before hatching, fish larvae were divided in to two different groups: 3 tanks with Skretting (SK) feed and 3 tanks with Otohime (OT) feed.

At an early stage of life it is vital to fulfill larval nutritional requirements as long as larvae develop. Following larval growth, different dimensions and nutritional values are adapted. The main components of the feeds used are described on the table 2.

Table 2 Main components of Skretting and Otohime feeds.

Product	Type	Size (µm)	Proteins (%)	Lipids (%)	Ash (%)	Fiber (%)	Phosphorus (%)
Skretting	150	100-200	59	14	14	0,2	2
	300	200-500	59	14	14	0,2	2
	0,5	565-650	62	14	8,5	0,2	1,4
Otohime	A2	150-250	53	8	16	3	1,5
	B1	250-360	51	11	15	3	1,5
	B2	360-650	51	11	15	3	1,5
	C1	580-840	51	11	15	3,5	1,5

Ingestion of microparticles is triggered by visual and chemical stimulation, therefore extrinsic characteristics might influence differently larvae appeal (Cahu and Infante, 2001). The feeds are very distinct from each other: Skretting is green while Otohime is orange; the texture on SK is more compact while OT is more loose; regarding their scent, SK is fishier and algae fragrance and OT has a shrimp fragrance.

Lumpfish larvae exhibit a sedentary behavior right after hatching, in order to ensure larvae ingestion it is essential to distribute feed in large excess to guarantee satiation. The feeding system was adapted accordingly to each feed and larval stage. On the third day post hatching, the tanks were hand fed which was spread on the surface once an hour on working times. This method was conducted during the following three days. After this period, the feed was placed in the feeder for 24h. All feedings transitions were made roughly at same time of growth for each feed. All transitions were carried out very

carefully, removing or infusing a new feed involves time in order for the fish to adapt. Insertion time guides are exhibited below on table 3.

Table 3 Guideline of first feeds in Nordland Rensefisk As.

Skretting	150	300	0,5	
Dph	3	20	70	
Otohime	A2	B1	B2	C1
Dph	3	20	50	80

2.2. Sampling procedures

Samples of 15 fish were taken, since hatching (day 0) following every second week for length and weight evaluation. On every sampling day, larvae were caught randomly using a plastic 3 ml pipette or a small net (on later stages). During larval phase, the fish were placed on a Petri dish, then anesthetized with MS 222 solution (Tricaine Methane Sulfonate; 300 mg/L), after the effect took place, the dishes were drained to facilitate handling.

- Weight

The larvae were then transferred from the Petri dish to a clean paper to absorb as much water as possible. Next, they were carefully set on a plate (previously tared) and weighted with 0,0001 g accuracy. During early juvenile phase, fish weight samples were taken by weekly average weight in the early juvenile hall department. A bucket is filled with water and tared; fish collected with a net are placed inside, weighted and counted back into the tank.

2.3. Estimated parameters

- Standard length (SL)

Larval length was measured since hatching up to the 10th week when all tanks are transferred to the early juvenile hall, early juvenile department. For length measurements, the Petri dish was placed over a graph paper (also known as grid paper) with squares of 10 x 10 mm. With the aid of a tweezer the larvae were placed horizontally and checked by magnifying glass. SL was measured in mm along the midline of the body from the tip of the snout to the end of the notochord.

- Mortality

The mortality in all tanks was registered daily throughout the whole experiment. The mortality of an individual sampling day is expressed in percentage, calculating the number of fish stock in the tank on that exact day, using the formula below:

$$\text{Mortality rate [\%]} = \frac{\text{initial number of larvae in each tank} - \text{number of larvae survived to the next sampling day}}{\text{initial number of larvae in each tank}} \times 100$$

3. Results

3.1. Average weight

Respectively to larval period, the growth on the first two weeks was quite even with SK: 0,0057±0,0500 g and OT: 0,0043±0,0740 g. From that point on until the 4th week, SK takes advantage with 0,0117±0,0500 g and OT: 0,0076±0,0740 g. However, notorious difference was found from the 4th up to the 6th week with SK: 0,0349±0,0500 g and OT: 0,0164±0,0740 g (Fig. 25).

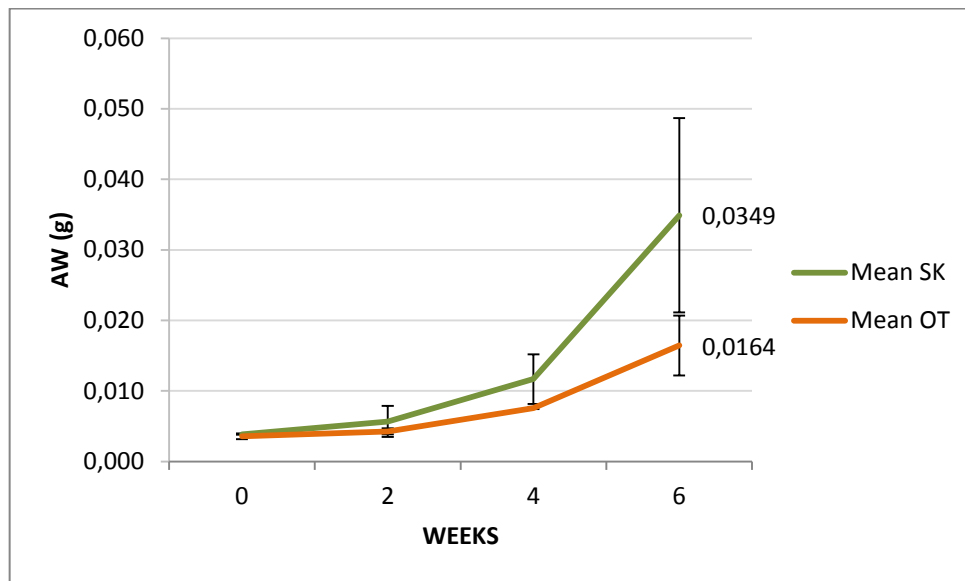


Fig. 25 Average weight of *C. lumpus* during larval development for Skretting and Otohime groups. Data are presented as mean±SD.

Since the 8th week both groups have shown very distinct growth speed as SK: 0,0809±0,0500 g to 0,57±0,0500 g and OT: 0,0453±0,0740 g to 0,1740±0,0740 g up to the 10th week. Even though SK showed a slower rhythm after the beginning of the 10th week, it recovered reaching 0,9667±0,0500 g on the 12th week, contrasting the 0,4233±0,0740 g of OT.

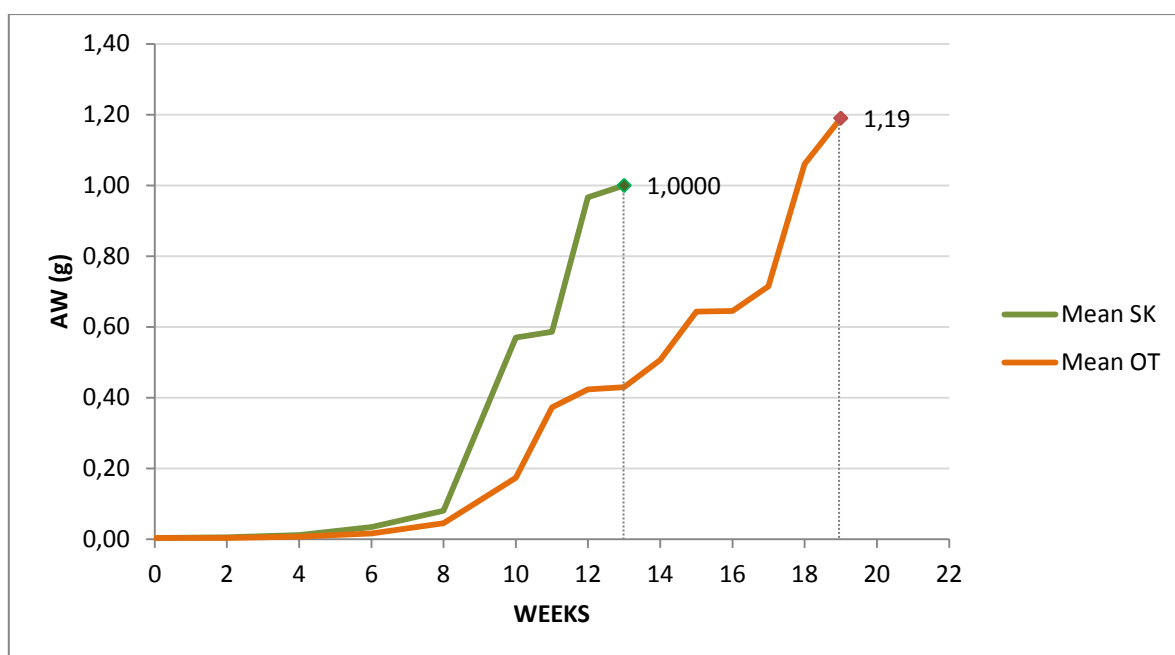


Fig. 26 Larval and early juvenile *C. lumpus* growth until 1 g for Skretting and Otohime groups. Data are presented as mean.

On week 13th SK reach the goal with $1,0 \pm 0,0500$ g on average, on the other hand, OT takes until week 19th to be $1,06 \pm 0,0740$ g (Fig. 26).

3.2. Standard length (SL)

In the beginning, larvae were growing at a gradual rhythm until the 4th week. From that point on SK starts to slightly increase, reaching 2 ± 1 mm of difference from OT between weeks 6th - 8th and finally with $5 \pm 0,65$ mm apart on the 10th week. To be noted the fact that SK's growth rate always seems to be ahead, since hatching (Fig. 27).

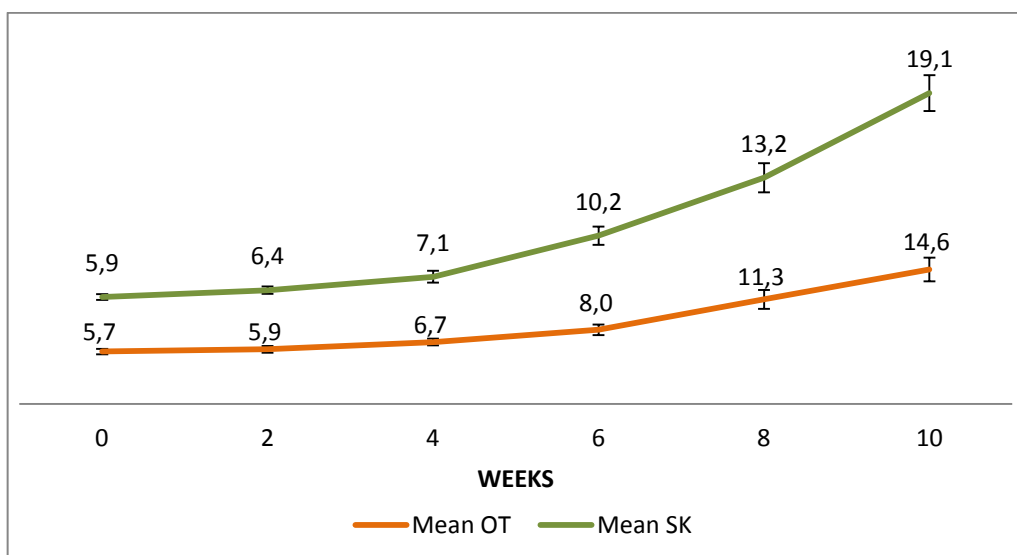


Fig. 27 Standard Length (mm) of *C. lumpus* during larval development for Skretting and Otohime groups. Data are presented as mean \pm SD.

3.3. Mortality

Dead larvae were counted every day from each tank and expressed as percentage from the initial survival number. Mortality rates were monitored from hatching until 1 g of weight for all tanks of each feeding group.

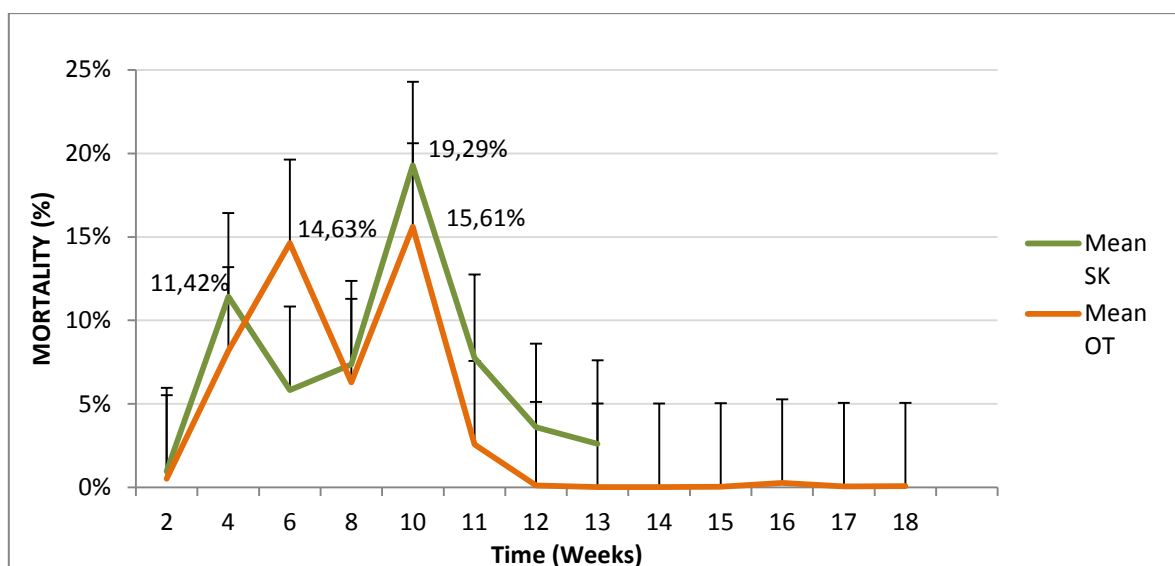


Fig. 28 Mortality rates of *C. lumpus* from hatching until 1 g for SK and OT group feedings. Data are presented as mean \pm SD.

Both groups exhibited two high peaks of mortality. The first peak of SK was the 4th week while OT was only on the 6th week with just 3% more. The two groups showed a pronounced drop of mortality on the following two weeks right after the first peak. The mortality starts to rise again, reaching the second peak at the same time for both groups

at the 10th week. SK attained the highest value with 19±7,37 % while OT highest value was 15±12,9 %. The mortality starts to descend until the end of the trial reaching less than 5% since week 12th (Fig. 28).

In overall perspective, SK group's evidence to have slightly higher mortality than OT with 10% more as it is seen on the Fig. 29.

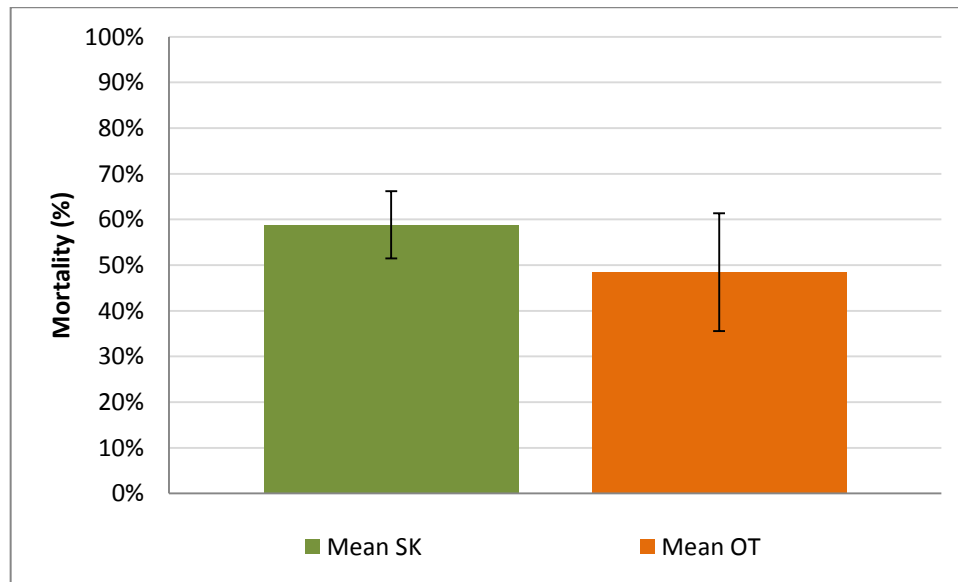


Fig. 29 Sum of mortality from hatching until 1 g for SK and OT feeding groups. Data are presented as mean±SD.

4. Discussion

Information concerning the effects of nutrition on development and survival of larvae and early juvenile of lumpfish is limited. However, it has been suggested that during early development stages in lumpfish larvae, the growth performance can be influenced by an optimal diet, improving development and survival rating during weaning process (Planas and Cunha, 1999; Moksness *et al.*, 2004; RCN, 2009; Southgate, 2012).

To test Nordland Rensefisk's assignment on which is the best feed to enhance larval growth in an industrial environment, one of the parameters taken into account was the average weight. During the larval phase on the first two weeks post-hatching both groups exhibit a natural growth. Differences start to appear on the following weeks where SK takes a notable advantage over OT, on the 6th week of the larval phase reaching double the weight comparing to OT. From the 8th week on SK has a remarkable growth, reaching 1 g on the 13th week, much sooner than OT, which only obtained it five weeks later on the 18th week. A similar trial on a larval phase between SK, OT and other commercial feeds

was performed on Florida pompano (*Trachinotus carolinus*) showing a similar result, having SK coming upfront until the 28th dph and OT displaying the lowest growth (Hauville *et al.*, 2014). This might be explained by the nutritional values which each feed is composed by thus having a different impact on the development of the digest system. Each diet has a different main source of protein, such as fish meal as a main predominant ingredient in Skretting Gemma Diamond 150 and krill in Otohime A. Micro diets have to provide adequate amino acid profile since the factor growth consists mainly of protein, as well as it needs to be highly digestible since marine fish larvae have to switch from a primary mode of digestion to an adult mode (Cahu and Infante, 2001; Moksness *et al.*, 2004; Bonaldo *et al.*, 2011; Hauville *et al.*, 2014). However, according to studies, both feeds are in the optimal range of 50-60% compound level (Cahu and Infante, 2001). Regarding to the lipid content, which defines the energy level being the main source of larval development, presenting a huge influence on growth, survival rates, resistance to stress and lower number of deformities (Mai *et al.*, 2009). Lipid compound is usually included in diets in high percentages as also several studies have proved that the higher the level the better performance is achieved (Cahu and Infante, 2001; Hauville *et al.*, 2014). Nevertheless, exceptions are verified, for example, according to Cahu and Infante (2001) who performed a study on a sea bream and concluded that the best level was 18% instead of 13, 23 or 27%, on the other hand, sea bass was most successful at 30%. Comparing the feeds of this trial might show why the larval performance shows such a distinctive growth rate between each other. The SK group achieved a growth rate of 145% where the OT group only achieved a 70% growth rate five weeks later. Regarding the levels of lipids in each feed, SK has the same content all along, 14%, while otohime in larval development has 8% then increases to 11% on the rest of the period, being always underlined by SK.

Our results concerning standard length revealed that on the moment of hatching (week 0) larvae were with expected size according to several references, 4-6 mm of length (Davenport, 1985; Benfey and Methven, 1986; Schaer and Vestvik, 2012) being acknowledge to be large larvae than other marine species (Brown, 1986) . A disparity starts to be noticed only on the 6th week when SK group takes the lead until the end of the period. Similar results were found by Hauville *et al.* (2014), once more, SK group was significantly larger after 22 dph comparing to the rest of the groups, exhibiting the same trend of growth even after 28 dph.

Survival during the experience indicated two critical peaks. The first one occurs between the 4th-6th week, although, SK groups presented the peak of mortality two weeks earlier

than OT. High mortality in the beginning of larval phase could be explained by the start of weaning process or by sub-optimal environment conditions (feeding pollution) in the tanks.

High mortality rates are common in early life stages in marine fish, also known as “critical period”, the initiation of exogenous feeding (Gallego *et al.*, 2012). It can be influence by innumerous factors: light, temperature, environment quality, size pellets, nutritional value, feed disposal, high density, larval development, among others (Rosenlund *et al.*, 1997; Planas and Cunha, 1999; Cahu and Infante, 2001; Gallego *et al.*, 2012; Rønnestad *et al.*, 2013). Information related to lumpfish larvae development is still scarce, nonetheless, it is established that lumpfish larva is well developed at hatching, with big eyes, well-formed ventral disk, notochord flexion under way, pectoral fins are functional, premaxilla, maxilla and dental are present (Davenport, 1985; Brown, 1986; Voskoboinikova and Kudryavtseva, 2014). To understand the reason of such a high mortality in the beginning, it was hypothesized that the feed size could not be adequate to mouth size. Fernández-Díaz *et al.* (1994) conducted a study on sea bream larvae where he observes that larvae select the size of ingested feed particles according their mouth width. Besides, he notes that larvae between 4.5 to 6 mm lengths had chosen particles in the range of 151 to 250 µm, and larvae with 6 mm length ingested particles over 250 µm. This result can serve as an analogy to lumpfish, once it is a marine larvae fully developed. Consequently, the feed particle sizes given during 4th to 6th weeks are both in an acceptable range for this phase.

The most pertinent parameter to evaluate survival is the weaning process. It is known the lumpfish larva have a yolk sack at hatching but in 15 dph it is no longer visible (Brown, 1986), which means, nutritional acquisitions are only dependent by ingestion of inert feed from that moment on. In this sense, adaption of fish larvae to microdiets require a period of morphologic, physiologic and behavior changes, besides early weaning being one of the possible reasons to delay the development of the digestive system (RCN, 2009; Pradhan *et al.*, 2014). These factors are probably the reason why high mortality was verified in the first place, however, it doesn't explain the reason why the mortality peaks were two weeks apart. Despite of both feedings being of similar size, the feeds display very different physical characteristics which might influence how much larvae ingested at a certain phase (Cahu and Infante, 2001; RCN, 2009). Additionally the feeds showed different pollution on the tanks. SK feed showed to be less tolerant to humidity, changing the texture faster than OT feed on the feeder, it was greasier on the surface of the walls resulting on larvae dispersal, being more susceptible to bacterial threats, plus requiring more effort on cleaning. On the other hand, OT feed showed to be much dryer, stable and easier to clear the bottom of the tanks. Poor environment on the tanks combined with

all factors above mentioned can be the answer why the SK group had the mortality peak earlier than the OT group.

The second peak of mortality is observed on the 10th week for both groups. This fact happened on the exact period when all groups were transferred to juvenile's tanks, between the 8th -10th week. Even though welfare is highly taken into account in all procedures and all co-workers are sensitized to be gentle in all operations, the actions taken can be rough for larvae. The procedure inevitably sets moments of stress, moreover if the larvae are not fully developed, or are lacking nutritional requirements, it is natural the most vulnerable end up dying. Additionally, the period of adaptation to a new environment with a stronger flow and higher dimension may justify the mortality noted (Moksness *et al.*, 2004; RCN, 2009).

Conclusions

This trial aimed to discover the main differences of two commercial feeds on early stages of lumpfish in intensive rearing. Essentially to determine which feed provided better growth, quality, performance and easier handling until fish could reach 1 g of weight.

The results of growth evaluation were remarkably different. The Skretting group showed a faster growth and development since 4th week. By means of average weight and length measurements, SK took a huge advantage reaching 1 g five weeks earlier than Otohime being 4 mm bigger. Analyzing mortality on larval phase it is likely to assume that both feeds did not fulfilled properly the nutritional values of lumpfish larvae as a start feed linking the time and mortality volume on the first high peak of mortality. Regarding to the second high peak of mortality, it is presumable all transferring procedure had a severe negative impact on larvae survival eliminating 15-20% of population.

After deliberate all results, Nordland Rensefisk concluded that Skretting feed would lead to more benefits in long term than Otohime feed. It is true that SK showed more volume of mortality and more pollution inside the tanks. However, the growth rate of SK redress the mortality verified on first growth stage. Besides new tactics are being implemented in order to minimize the impact on larvae transportation and better cleaning strategies. Consequently Skretting feed will be more liable.

The results of the present experiment could be useful to contemplate relevant details concerning commercial feeds in intensive rehearing panorama. However, to enrich the knowledge about lumpfish all areas require further research and deeper investigations to understand how to satisfy lumpfish needs in all growth stages and provide the best welfare as possible during the whole cycle. More and more, that lumpfish production is increasing every year with more efficiency.

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